12 Rec'd PCT/PTO 29 JUL 1998

FORM PTO-1390 (REV. 1-98) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY 'S DOCKET NUMBER FRIDKIN=1
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 CFR 1.5 09/117380
INTERNATIONAL APPLICATION NO. **PCT/IL97/00032**	INTERNATIONAL FILING DATE **January 27, 1997**	PRIORITY DATE CLAIMED **January 31, 1996**

TITLE OF INVENTION
ANTI-INFLAMMATORY PEPTIDES DERIVED FROM C-REACTIVE PROTEIN

APPLICANT(S) FOR DO/EO/US
Mattiyahu FRIDKIN et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☒ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

    1. **A courtesy copy of the specification as originally filed.**
    2. **A courtesy copy of the first page of the International Publication (WO97/28182).**
    3. **A courtesy copy of the International Search Report.**
    4. **Formal drawings, 2 sheets, figures 1-2.**

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO. PCT/IL97/00032	ATTORNEY'S DOCKET NUMBER FRIDKIN=1

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1070.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $930.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $790.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $720.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $98.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 98.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	13 - 20 =		x $22.00	$	
Independent claims	1 - 3 =		x $82.00	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 228.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28). +				$	
SUBTOTAL =				$ 228.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$ 228.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$ 228.00	
				Amount to be refunded:	$
				charged:	$

a. ☒ A check in the amount of $ 228.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. ______ in the amount of $ ______ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4035. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

BROWDY AND NEIMARK, P.L.L.C.
419 Seventh Street N.W., Suite 300
Washington, D.C. 20004

Roger L. Browdy
SIGNATURE

Roger L. Browdy
NAME

25,618
REGISTRATION NUMBER

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#2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Art Unit:
M. FRIDKIN et al.	)	
IA No.: PCT/IL97/00032	)	Washington, D.C.
IA Filed: January 27, 1997	)	
U.S. App. No.: (Not Yet Assigned)	)	July 29, 1998
National Filing Date: (Not Yet Received)	)	
For: ANTI-INFLAMMATORY...	)	Docket No.: FRIDKIN=1

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and prior to calculation of the filing fee, kindly amend as follows:

IN THE CLAIMS

Claim 8, line 1, delete "any one of claims 1 to 7", and insert therefor --claim 1--.

Claim 9, line 2, delete "any one of claims 1 to 8", and insert therefor --claim 1--.

Claim 10, line 1, delete "any one of claims 1 to 8", and insert therefor --claim 1--.

Claim 12, line 3, delete "any one of claims 1 to 8", and insert therefor --claim 1--.

REMARKS

The above amendments to the claims are being made in order to eliminate any properly multiply dependent claims, for the

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purpose of reducing the filing fee. Please enter this amendment prior to calculation of the filing fee in this case.

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicants

By ______________________
Roger L. Browdy
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Art Unit: #3
M. FRIDKIN et al.	)	
IA No.: PCT/IL97/00032	)	Washington, D.C.
IA Filed: January 27, 1997	)	
U.S. App. No.: (Not Yet Assigned)	)	July 29, 1998
National Filing Date: (Not Yet Received)	)	
For: ANTI-INFLAMMATORY...	)	Docket No.: FRIDKIN=1

SUPPLEMENTAL PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to examination on the merits, kindly amend as follows:

IN THE CLAIMS

Please delete claims 10 and 11 in their entirety.

REMARKS

Claims 1-9 and 12-13 presently appear in this case. The present amendments are being made in order eliminate the improper "use" claims.

In re of FRIDKIN=1

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicants

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Applicant or Patentee: Matityahu FRIDKIN et al. Attorney's Docket No.: FRIDKIN=1
Appln. or Patent No.: ____________ Filed or Issued: ____________
For: ANTI-INFLAMMATORY PEPTIDES DERIVED FROM C-REACTIVE PROTEIN

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL BUSINESS ENTITY STATUS
(37 CFR 1.9(f) and 1.27(C)) - SMALL BUSINESS CONCERN

I hereby declare that I am
[ ] the owner of the small business concern identified below:
[XX] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN YEDA RESEARCH AND DEVELOPMENT CO. LTD.
ADDRESS OF SMALL BUSINESS CONCERN P.O. Box 95, Rehovot 76100, Israel

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled ANTI-INFLAMMATORY PEPTIDES DERIVED FROM C-REACTIVE PROTEIN by inventors Matityahu FRIDKIN and Eran J. YAVIN described in:

[ ] the specification filed herewith with title listed as above.
[XX] application no. ____________, filed July 29, 1998.
[ ] patent no. ____________, issued ____________.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights in the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

*Note: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities (37 CFR 1.27).

NAME ____________
ADDRESS ____________
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION
NAME ____________
ADDRESS ____________
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

YEDA
RESEARCH AND DEVELOPMENT
CO. LTD.

NAME OF PERSON SIGNING Paulina Ben-Ami
TITLE OF PERSON SIGNING OTHER THAN OWNER Vice President
ADDRESS OF PERSON SIGNING P.O. Box 95, Rehovot 76100, Israel
SIGNATURE Paulina Ben-Ami DATE September 27, 1998

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09/117380

ANTI-INFLAMMATORY PEPTIDES DERIVED FROM C-REACTIVE PROTEIN

Field of the Invention

The present invention relates to synthetic peptides derived from the primary sequence of the acute phase reactant C-reactive protein (CRP), which peptides inhibit *in vitro* the enzymatic activities of human leukocyte elastase (hLE) and human leukocyte cathepsin G (hCG), two potent serine proteases associated with tissue damage occurring in the course of several chronic inflammatory conditions. The invention further relates to anti-inflammatory pharmaceutical compositions comprising said CRP-derived peptides.

Abbreviations:

The following abbreviations will be used throughout the specification:

CRP, C-reactive protein; **hLE**, human leukocyte elastase; **hCG**, human leukocyte cathepsin G; **α_1-PI**, α_1-protease inhibitor; **ACT**, α-antichymotrypsin; **MeOSuc-AAPV-NA**, methoxysuccinyl-Ala-Ala-Pro-Val-nitroanilide; **Suc-AAPF-NA**, succinyl-Ala-Ala-Pro-Phe-nitroanilide.

Background of the Invention

C-reactive protein (CRP) is a plasma protein classified as a major acute phase reactant due to its dramatic accumulation in the blood stream during the inflammatory response. Within a relatively short period (24-48 hr) following tissue injury or certain traumatic events, the CRP blood concentration may rise 1000-fold over the normal level to as high as 1 mg/mL (Ballue and Kushner, 1992).

CRP consists of five identical sub-units that contain each 206 amino acids bridged by a single disulfide bond and that aggregate non-covalently into a cyclic pentamer termed pentraxin. The precise biochemical function of CRP as a whole entity is still obscure. CRP

was shown to bind to specific receptors on human neutrophils (K_d ~$5x10^{-8}$ M), monocytes (K_d~10^{-7} M), and other inflammatory-related cells *in vitro* (Ballue and Kushner, 1992).

In the laboratories of the present inventors and their collaborators it was found that following binding to neutrophils, CRP is subsequently degraded by a membrane-associated neutral serine protease, which has been characterized (Shephard et al., 1992), and by lysosomal-derived enzymes, to yield various low molecular weight peptides. Several of these peptides were identified, synthesized, and shown to be potent anti-inflammatory agents inhibiting neutrophil phagocytosis, degranulation, and superoxide ion (O_2^-) generation (Shephard et al., 1990; Yavin et al., 1995). Superoxide ion is the parent compound of several destructive mediators that are believed to play a central role in inflammation-associated tissue injury (Ballue and Kushner, 1992).

The most prominent of the peptides disclosed by Shephard et al., 1990, and Yavin et al., 1995, were derived from within the primary sequence of CRP as follows: Asp_{70}-Ile-Gly-Tyr-Ser_{74} , Lys_{201}-Pro-Gln-Leu-Trp-Pro_{206} , Leu_{83}-Phe-Glu-Val-Pro-Glu-Val-Thr_{90} , Val_{77}-Gly-Gly-Ser-Glu-Ile_{82} (Shephard et al., 1990) and Asn_{160}-Met-Trp-Asp-Phe-Val_{165} , Gln_{203}-Leu-Trp-Pro_{206} , Ser_{18}-Tyr-Val-Ser-Leu-Lys_{23} (Yavin et al., 1995). These peptides were shown by the authors to inhibit neutrophilic function, indicating that they may be capable of regulating superoxide ion production by neutrophils *in vivo* during the acute phase response as part of a complex protective mechanism. However, as shown in the examples of the present application, several of these peptides and additional peptides with close proximity within the primary sequence of CRP have no hLE inhibitory capability.

Human leukocyte elastase (hLE) and human leukocyte cathepsin G (hCG) are potent neutral serine proteases found in the azurophilic granules of neutrophils, which are involved in the intracellular digestion of proteins and play an important role in phagocytosis and host defense against invading organisms. In the extracellular environment, hLE is capable of degrading various connective tissue proteins including highly cross-linked elastin whereas hCG is very effective in degrading proteoglycans and collagens and has been shown to augment the elastolytic capability of hLE (Groutas, 1987).

The release of enzymes into the extracellular medium by activated neutrophils is normally controlled by several potent inhibitors. The most specific natural inhibitors, α_1-protease inhibitor (α_1-PI) and α-antichymotrypsin (ACT), are directed against hLE and hCG, respectively (Groutas, 1987). Imbalances in the levels of tissue proteases such as hLE and hCG, and their inhibitors, allow excess hLE and hCG to attack connective tissue, and are implicated in the severe and permanent tissue damage associated with pulmonary emphysema (Groutas, 1987), rheumatoid arthritis (Gallin et al., 1988), cystic fibrosis (Jackson et al., 1984) and several other inflammatory conditions. Major research efforts have been dedicated to develop potent inhibitors of hLE and hCG based on a wide variety of low molecular weight organic compounds (Edwards and Bernstein, 1994) such as 3,3-dialkylazetidin-2-ones, proposed as orally active β-lactam inhibitors of hLE (Finke et al., 1995).

CRP as a whole protein was reported to have no inhibitory effect on hLE (Vachino et al., 1988). In contrast, novel biologically active CRP-derived peptides, previously concealed within the inner hydrophobic region of each subunit, have been found, in accordance with the present invention, to significantly inhibit the enzymatic activities towards destructive enzymes.

Summary of the Invention

The present invention relates to synthetic CRP-derived peptides, which inhibit *in vitro* the enzymatic activity of hLE and hCG.

In particular, the present invention relates to a synthetic peptide capable of inhibiting *in vitro* the enzymatic activity of human leukocyte elastase (hLE) and/or of human cathepsin G (hCG), said peptide being selected from:

(i) a core peptide corresponding to positions 89-96 of the sequence of human C-reactive protein (CRP) of the formula:

$$Val_{89}\text{-Thr-Val-Ala-Pro-Val-His-}Ile_{96}$$

or a modification thereof characterized by:

(ii) substitution of Ile_{96} by a hydrophobic amino acid residue ;

(iii) substitution of His_{95} by D-His or by a residue selected from Asp, Glu, Ser, Thr, Phe and Tyr, N-alkyl derivatives thereof and D-forms of the foregoing;

(iv) substitution of Val94 by D-Val or by a residue selected from Ala, His and Phe, and D-forms of the foregoing;

(v) substitution of Ala92 by a hydrophobic amino acid residue;

(vi) substitution of Val91 by Ala or Gly;

(vii) substitution of Thr90 by a residue selected from Asn, Asp, Gln, Glu, Ala, Val and Pro;

(viii) substitution of Val89 by a hydrophobic amino acid residue;

(ix) a peptide obtained by elongation of a peptide (i) to (viii) at the N- and/or C-terminal;

(x) an amide of the C-terminal of a peptide (i) to (ix); and

(ix) an N-acyl derivative of a peptide (i) to (x).

The invention further relates to anti-inflammatory pharmaceutical compositions comprising a CRP-derived peptide of the invention and a pharmaceutically acceptable carrier.

In another aspect, the invention relates to a method of treatment of an inflammatory disorder, e.g. rheumatoid arthritis, pulmonary emphysema, cystic fibrosis and other chronic inflammatory condition, which comprises administering to a patient in need thereof an effective amount of a CRP-derived peptide according to the invention.

Brief Description of the Drawings

Fig. 1 depicts the sequence of the human C-reactive protein (CRP).

Fig. 2 is a graph of the RP-HPLC chromatograms of the degradation profile of the CRP-derived core peptide **1** of the sequence Val89-Thr-Val-Ala-Pro-Val-His-Ile96 by hLE at several time intervals.

Detailed Description of the Invention

The present invention provides a series of synthetic peptides derived from the sequence of CRP and to pharmaceutical compositions comprising the peptides which are anti-inflammatory by inhibiting either hLE or hCG activity, or both. These biologically

active peptides can be used to inhibit hLE and/or hCG and thereby have utility in controlling tissue damage associated with chronic inflammation.

A careful examination of the sequence of CRP reveals a specific region within the protein's sequence which is similar, though not identical, to the active site of α_1-PI, the natural inhibitor of hLE, as shown below:

CRP: **Ser-Phe**-Thr-Val-Gly-**Gly-Ser-Glu-ILe-Leu**-Phe-Glu-**Val-Pro**-Glu-

α_1-PI **Thr-Ile**-Asn-Glu-Lys-**Gly-Thr-Glu-Ala-Ala**-Gly-**Ala-Met**-Phe-

CRP: **Val-Thr-Val-Ala-Pro-Val** $_{94}$ ⇔**His-Ile**-Cys-s-s-Cys-**Leu-His-Phe**

α_1-PI **Leu-Glu-Ala-Ile-Pro-Met**$_{358}$⇔**Thr-Ile**-Pro-Pro-Glu-**Val-Lys-Phe**

The long range sequence match between CRP and α_1-PI is shown. Bold letters denote similar amino acids, with respect to approximate steric volume, hydrophobicity and charge, or identical amino acids. From the carboxy terminal of peptide **1** (underlined) the sequence similarity is apparent although shifted from a certain position by one amino acid (e.g. Gly$_{79}$-Ser-Glu-ILe-Leu$_{83}$ in CRP vs. Gly$_{344}$-Thr-Glu-Ala-Ala$_{348}$ in α_1-PI). From the amino terminal, sequence similarity may be observed through the single disulfide bridge (represented by -s-s-) found in CRP. The cleavage site (symbol ⇔) in α_1-PI is the Met$_{358}$-Thr$_{359}$ bond and in CRP-based peptide inhibitors is theoretically at the Val$_{94}$-His$_{95}$ bond.

The core peptide **1**, Val89-Thr-Val-Ala-Pro-Val-His-Ile96, was chosen due to its similarity with the active site of the natural inhibitor of elastase: α_1-PI. This sequence contains the highest ratio of similar vs. dissimilar amino acids. Based on the X-ray crystallographic data obtained for hLE complexed with Turkey ovimcoid inhibitor (Bode et al., 1989), the preferred amino acid required by each subsite of hLE was formulated with respect to CRP derived analogs. For example, the enzyme's main hydrophobic pocket, in which CRP's Val94 is accommodated, is large enough to contain large hydrophobic amino acids such as Ile or Leu, yet it is not large enough to contain Phe which hCG actually prefers. This core peptide **1** is cleaved specifically at the Val-His bond, making it an ideal candidate for further subsite modifications and L to D amino acid replacements.

Based on the results obtained for the core peptide **1**, additional peptides **2-23** were synthesized (Table 1).

According to the invention, the residues Val_{89}, Ala_{92} and Ile_{96} of the core peptide **1** may be replaced by a residue of a natural aliphatic or aromatic hydrophobic amino acid, such as Leu, Ile, Val, Phe or Tyr, or of a non-natural hydrophobic amino acid, such as norleucine (Nle) and norvaline (Nva).

The residue His_{95} may be replaced preferably by an aromatic amino acid, such as Phe or Tyr, or by Asp, Glu, Ser or Thr. D-amino acid modifications, e.g. (D)His, and N-alkylation of the peptide bond, are most beneficial in this position to prevent peptide cleavage by the enzyme.

The residue Val_{94} is the preferred residue at this position directed towards hLE inhibition, while specificity towards hCG is gained by aromatic amino acid substitution such as Phe or His. D-amino acid modifications, e.g. (D)Val, (D)Ala, (D)Phe and (D)His are most beneficial in this position to prevent peptide cleavage by the enzyme.

Proline is important in creating a bond to the stretched, open chain peptide which increases its specific fit into the binding site of hLE and hCG (Bode et al., 1989). If the residue Pro_{93} is replaced in this position, binding may occur in different orientations of the peptide which dramatically reduces its inhibitory activity. Thus attempt to replace it by sarcosine (i. e. N-methyl-glycine that bears some chemical resemblance to Pro, see peptide **30** in Table 2) led to a dramatic loss in inhibitory activity as compared to the core peptide **1**. It is therefore not advisable to modulate this position.

The residue Thr_{90} may be replaced by Asn, Asp, Gln or Glu, Pro, or by a medium sized hydrophobic amino acid, such as Ala or Val.

Elongation of the peptide chain of the core peptide **1** or of a modified core peptide obtained by substitution of one or more amino acid residues as described above, leads to augmentation of inhibitory activity both towards hLE (see peptides **2, 3, 3a, 3b, 3c, 4** and **5**) and towards hCG (see peptides **2, 3, 3a, 3b** and **3c**). The C-terminus addition of Cys_{97}-Thr_{98} to peptide **3** increases inhibitory activity towards both enzymes (peptide **3a**) while the additional C-terminus fragment, Ser_{99}-Trp-Glu-Ser-Ala_{104}, decreases inhibitory activity towards both enzymes (peptide **3b**). In contrast, inhibitory activity towards hCG is totally abolished by elongation through the cystein disulfide bond (see peptides **4** and **5**). A dramatic rise in hLE and hCG inhibitory activity is observed when peptide **3a** is elongated towards the N-terminus by the additional amino acids Asp_{70}-Ile-Gly-Tyr_{74} (peptide **3c**).

Deletion of amino acid residues from both the amino and carboxy terminals leads to dramatic reduction of inhibitory activity towards hLE in comparison to the core peptide (see peptides **25** and **26** in Table 2)

Amides ($CO\text{-}NH_2$) of the carboxy terminal of the peptides of the invention show augmented inhibitory activity towards hLE in comparison with the core peptide **1**.

N-acyl derivatives of the N-terminal have shown augmented inhibitory activity towards hCG in comparison with the core peptide **1**. Examples of these acyl derivatives correspond to the formula R-X-CO- wherein R is a substituted or unsubstituted hydrocarbyl, preferably alkyl or aryl, and X is a covalent bond, O, NH or NHCO. Examples of acyl radicals are octanoyl, monomethoxysuccinyl, acetylaminocaproyl, adamantyl-NH-CO-, and more preferably, carbobenzoxy (i.e. benzyl-O-CO-), naphthyl-NH-CO-, and Fmoc (i.e. fluorenylmethyl-O-CO-),.

Preferred CRP-derived peptides according to the invention are the core peptide **1**; peptides obtained by substitution of His_{95}, e.g. by Phe (peptide **10**); peptides obtained by elongation of peptide **1** at the amino and/or carboxy terminals and amides thereof, such as the peptides **2**, **3**, **4**, **5**, **12**, and **14**; and N-acyl derivatives of peptide **1**, such as the peptides **16**, **18**, **21** and **23**.

The peptides of the invention are prepared by standard methods for the synthesis of peptides. In one embodiment of the invention, the peptides are prepared as set forth in the Examples hereinbelow.

In another aspect, the present invention relates to pharmaceutical compositions comprising a peptide of the invention and a pharmaceutically acceptable carrier. The compositions are prepared by well-accepted methods for preparation of peptide-containing pharmaceutical compositions for administration in a suitable form, e.g. orally, subcutaneously, intranasal, and parenterally including intravenous, intramuscular and intraperitoneally, according to the inflammatory condition to be treated.

In a further aspect, the invention relates to a method of treatment of a chronic inflammatory condition which comprises administering to a patient in need thereof an effective amount of a peptide according to the invention. Examples of such chronic inflammatory conditions are rheumatoid arthritis, pulmonary emphysema and cystic

fibrosis. The anti-inflammatory peptide is administered and dosed in accordance with good medical practice, taking into consideration the clinical condition of the patient, the site and method of administration, schedule of administration and other factors known to medical practicioners.

The invention will now be illustrated by the following non-limiting examples.

EXAMPLES

Materials and Methods

(i) General Solid Phase Peptide synthesis: Peptides were prepared by conventional solid phase peptide synthesis, with ABIMED AMS-422 automated solid phase multiple peptide synthesizer (Langenfeld, Germany). The Fmoc-strategy (Fmoc=9-fluorenyl-methoxycarbonyl) was used through peptide chain assembly, following the company's commercial protocols. In each reaction vessel, 12.5 μmol of Wang resin was used which contained the first, covalently bound, corresponding N-Fmoc C-terminal amino acid (typical polymer loadings of 0.3-0.7 mmols/g resin were employed). Fmoc deprotection was achieved using duplicate flushes with 20 % piperidine in dimethylformamide (DMF), typically for 10-15 min at room temperature, depending on the length of peptide and Fmoc- protected amino acid type, as given by the company's protocols.

Side chain-protecting groups were tert.-butyloxycarbonyl (*t.*-Boc) for Lys, diaminobutyric-acid (DAB), and Trp; trityl (Trt) for Asn, Cys, Gln, His, and (D)-His; tert.-butyl-ester (O-*t*-But) for Asp and Glu; tert.-butyl-ether (*t*-But) for Ser, Thr, and Tyr; 3-nitro-2-pyridinesulfenyl (NPYS) for Cys in the synthesis of peptides **4** and **5**; and carbobenzoxy (Cbz) for the N-terminus amino acids Val and Phe in the synthesis of peptides **20** and **21**, respectively.

Coupling was achieved, as a rule, using two successive reactions with 50 μmol (4 eqv.) of corresponding N-Fmoc protected amino acid, 50 μmol (4 eqv.) of PyBop reagent (benzotriazole-1-oxytris-pyrrolidino-phosphonium-hexafluoro-phosphate), and 100 μmol (μeqv.) of 4-methyl-morpholine (NMM), all dissolved in DMF, typically for 20-45 min at

room temperature, depending on the length of peptide and amino acid derivative type, as given by the company's protocols.

Cleavage of the peptide from the polymer was achieved by reacting the resin with trifluoroacetic acid/H_2O/triethylsilane (TFA/H_2O/TES; 90/5/5; v/v) for 1 to 2 hours at room temperature. The crude unprotected peptides were then cooled down to 4°C, precipitated with ice-cold di-tert.-butylether (DTBE) and centrifuged for 15 min, 3000 RPM at 4°C. The pellet was washed and centrifuged 3 times with DTBE, dissolved in 30 % acetonitrile in H_2O, and lyophilized.

All protected amino acids, coupling reagents, and polymers were obtained from Nova Biochemicals; Läufelfingen, Switzerland. Synthesis-grade solvents were obtained from Labscan; Dublin, Ireland.

(ii) Reversed-phase high performance liquid chromatography (RP-HPLC): Synthetic peptides were purified by using a prepacked LiChroCart RP-18 column (250x10 mm, 7 μm bead size), employing a binary gradient formed from 0.1 % TFA in H_2O (solution A) and 0.1 % TFA with 25 % H_2O in acetonitrile (solution B), eluted at t=0 min B=5 % t=5 min B=5 % t=60 min B=70 % (flow-rate 5 mL/min). Analytical RP-HPLC was performed using a prepacked Lichrospher-100 RP-18 column (4x250 mm, 5 μm bead size) using the same buffer system (flow-rate 0.8 mL/min). All peptide separations were performed using a Spectra-Physics SP8800 liquid chromatography system equipped with an Applied Biosystems 757 variable wave-length absorbence detector. The column effluents were monitored by UV absorbence at 220 nm, and chromatograms were recorded on a Chrome-Jet integrator. Following HPLC purification, the lyophilized peptides (generally > 90 % pure for crude samples after synthesis as described below) were purified to > 97 %. All solvents and HPLC columns were obtained from Merck; Darmstadt, Germany.

(iii) Amino acid composition analysis: Purified peptide solutions were roto-evaporated (≈40 μg of peptide in 40 μL solution with 5 μg of norleucine as an un-natural amino acid internal standard), hydrolyzed in 6 N HCl at 110 °C for 22 hours under vacuum and analyzed with a Dionex amino acid analyzer. This quantification was used as a basis for determination of the total yield of peptide. Several of the peptides synthesized were

analyzed by Liquid Secondary-ion Mass-spectrometry which confirmed their expected $(M+H)^+$, protonated molecular ions.

(iv) Isolation of hLE and hCG: The isolation of neutrophilic enzymes was based on the two-step aprotinin-sepharose affinity chromatography and carboxymethyl-cellulose (CMC) ion exchange chromatography (Heck et al., 1985). Neutrophils (1.4 billion) were isolated from whole blood obtained from a single healthy laboratory donor by dextran sedimentation and Ficoll/hypaque gradient centrifugations as described elsewhere (Metcalf et al., 1986). The enzymatic activity was assayed with MeOSuc-AAPV-NA for hLE determination and Suc-AAPF-NA for hCG determination (both in 100 mM Hepes buffer, pH 7.4, containing 0.05 % of the anionic detergent Brij-35). The activities of the individual enzymatic fractions were 100 % free from cross-contamination. The step-wise elution profile on the CMC column with a long 0.45 M NaCl elution step (20 column volumes) afforded the effective separation between the two enzymes. The fractions containing hLE and hCG were dialyzed each against 0.1 % pyridinium acetate, pH 5.3, divided into 20 aliquots, lyophilized, and stored at -20 °C until use. By the initial rates of reactions and the known values of K_{cat} (hLE= 54 μM, hCG= 2900 μM) and K_m (hLE= 13.3 $sec.^{-1}$, hCG= 3.1 $sec.^{-1}$), the amount of enzyme was estimated to be approximately 15 μg/aliquot for hLE and 12 μg/aliquot for hCG, such values being confirmed by active site titration with α_1-PI and ACT.

(v) Inhibition experiments with hLE: Peptides were dissolved in 100 mM Hepes buffer pH 7.4 containing 0.1 % Brij-35 with 10 % DMSO to yield 600 μM solutions, which were used to make further dilutions with the same buffer, and 80 μL aliquots were added in duplicates to 96-well plates. The substrate, 600 or 900 μM MeOSuc-AAPV-NA in the same buffer with 5 % DMSO, was added to each well in addition to the blank wells, and the plate was placed in the plate reader equilibrated to 37 °C (Dynateck MR-6000). Lyophilized aliquots of hLE were dissolved in 1600 μL of 100 mM Hepes buffer without DMSO, and 80 μL of the enzyme solution was added to the peptides and substrate to initiate the reaction. The kinetics program read the plate at 405 nm every 2 min for 20 min (with a 3 sec shaking period between readings), and plotted the results as well as the average of each duplicate. The final volume was 240 μL containing: 5 % DMSO, 1-200 μM of peptide, 200 μM substrate, and 0.75 μg (about 25 picomol) enzyme.

(vi) Inhibition experiments with hCG: Similar conditions to hLE inhibition experiments were used except the substrate: 80 μL of 1.8 mM Suc-AAPF-NA. The enzyme was dissolved in 800 μL buffer, and the reaction was monitored every 6 min for 1 hour. The final volume was 240 μL containing: 5% DMSO, 1-500 μM peptide, 600 μM substrate, and 1.2 μg enzyme (about 40 picomol).

(vii) Degradation profiles of peptides by RP-HPLC: Several active peptide inhibitors were dissolved in calcium- and magnesium-free phosphate-buffered saline (PBS), 125 μg /250 μL, mixed with 0.25 aliquots of hLE or hCG in 250 μL PBS and incubated at 37 °C. Periodically (1,3,8, and 24 hour), 100 μL samples were removed from the reaction vessel. The samples were diluted with 150 μL of 0.1 % TFA, frozen with liquid nitrogen, and stored at -20 °C prior to HPLC analysis.

(viii) Calculations: For hLE, V is determined by fitting a linear equation to the first 6 time-points (10 min) of the kinetics data using the least squares method. Without exception, all R^2 factors were > 0.998. Several inhibitor concentration (250, 375, and 500 μM) in duplicates and two control wells were used to fit a linear equation to graphs of Vo/V_i-1 vs. [I] for each inhibitor using the least squares method (8 data points for each inhibitor). From calculating the error in the slope of the equation, the relative error for K_i was deduced:

$K_i= \{slope*(1+[S]/K_m)\}^{-1}$ because $K_i= [I]*\{(1+[S]/K_m)*(Vo/V_i-1)\}^{-1}$.

For hCG, V is determined by fitting a quadratic equation to the total kinetic data (60 min), using the least squares method and calculating V at t=0. Without exception, all R^2 factors were > 0.996. Two inhibitor concentrations (250 and 500 μM) in duplicate and two control wells were used for each inhibitor, and in a similar fashion to hLE, K_i was deduced (6 data points for each inhibitor).

Example 1: Synthesis of core peptide 1 and other peptides

The sequence of the peptides **1-23** according to the invention and the inhibition constants (Ki) of human hLE and human hCG are shown in Table 1. The sequences of the comparison peptides **24-30** and the hLE Ki are shown in Table 2. The amino acid analysis of peptides **1-30** is shown in Table 3.

Table 1

CRP-derived peptides of the invention and inhibition constants (Ki) of human leukocyte elastase (hLE) and human cathepsin G (hCG).

Peptide	Sequence	hLE Ki (μM)	hCG Ki (μM)
1	Val89-Thr-Val-Ala-Pro-Val-His-Ile96	120 ± 15	1400 ± 200
2	Gly79-Ser-Glu-Ile-Leu-Phe-Glu-Val-Pro-Glu-Val-Thr-Val-Ala-Pro-Val-His-Ile96	50 ± 5	1200 ± 200
3	Ser74-Phe-Thr-Val-Gly-Gly-Ser-Glu-Ile-Leu-Phe-Glu-Val-Pro-Glu-Val-Thr-Val-Ala-Pro-Val-His-Ile96	27 ± 3	500 ± 100
3a	Ser74-Phe-Thr-Val-Gly-Gly-Ser-Glu-Ile-Leu-Phe-Glu-Val-Pro-Glu-Val-Thr-Val-Ala-Pro-Val-His-Ile-Cys-Thr98	20 ± 3	180 ± 30
3b	Ser74-Phe-Thr-Val-Gly-Gly-Ser-Glu-Ile-Leu-Phe-Glu-Val-Pro-Glu-Val-Thr-Val-Ala-Pro-Val-His-Ile-Cys-Thr-Ser-Trp-Glu-Ser-Ala103	22 ± 4	200 ± 30
3c	Asp70-Ile-Gly-Tyr-Ser-Phe-Thr-Val-Gly-Gly-Ser-Glu-Ile-Leu-Phe-Glu-Val-Pro-Glu-Val-Thr-Val-Ala-Pro-Val-His-Ile-Cys-Thr98	4.0 ± 0.6	22 ± 3.0
4	Val89-Thr-Val-Ala-Pro-Val-His-Ile-Cys97-Cys36-His-Leu-Phe39	85 ± 5	N.I.
5	Gly79-Ser-Glu-Ile-Leu-Phe-Glu-Val-Pro-Glu-Val-Thr-Val-Ala-Pro-Val-His-Ile-Cys97-Cys36-His-Leu-Phe39	55 ± 5	N.I.
6	Val89-Thr-Val-Ala-Pro-Val-**(D)His**-Ile96	450 ± 45	N.I.
7	Val89-Thr-Val-Ala-Pro-**(D)Val**-His-Ile9	330 ± 130	N.I.
8	Val89-Thr-Val-Ala-Pro-**(D)Val**-**(D)His**-Ile96	490 ± 50	W.I.
9	Val89-Thr-Val-Ala-Pro-Val-Ser-Ile96	200 ± 20	N.I.

10	Val$_{89}$-Thr-Val-Ala-Pro-Val-**Phe**-Ile$_{96}$	110 ± 25	1.0 ± 0.2
12	Val$_{89}$-Thr-Val-Ala-Pro-Val-His-Ile$_{96}$-**Pro-NH$_2$**	70 ± 5	W.I.
13	Val$_{89}$-Thr-Val-Ala-Pro-**Phe**-His-Ile$_{96}$-**Pro-NH$_2$**	180 ± 30	900 ± 200
14	Val$_{89}$-Thr-Val-Ala-Pro-Val-His-Ile$_{96}$-**Pro-Pro-NH$_2$**	85 ± 10	W.I.
15	**MeOSuc**-Val$_{89}$-Thr-Val-Ala-Pro-Val-His-Ile$_{96}$	160 ± 30	W.I.
16	**MeOSuc-Phe**-Val$_{89}$-Thr-Val-Ala-Pro-Val-His-Ile$_{96}$	100 ± 10	100 ± 200
17	**Adamantyl**-NH-CO-Val$_{89}$-Thr-Val-Ala-Pro-Val-His-Ile$_{96}$	130 ± 15	W.I.
18	**Naphtyl**-NH-CO-Val$_{89}$-Thr-Val-Ala-Pro-Val-His-Ile$_{96}$	240 ± 35	300 ± 40
19	**Octanoyl**-Val$_{89}$-Thr-Val-Ala-Pro-Val-His-Ile$_{96}$	280 ± 25	W.I.
20	**CBz**-Val$_{89}$-Thr-Val-Ala-Pro-Val-His-Ile$_{96}$	165 ± 35	N.I.
21	**CBz-Phe**-Val$_{89}$-Thr-Val-Ala-Pro-Val-His-Ile$_{96}$	380 ± 115	430 ± 60
22	**Acetyl-aminocaproyl**-Val$_{89}$-Thr-Val-Ala-Pro-Val-His-Ile$_{96}$	210 ± 30	W.I.
23	**Fmoc**-Val$_{89}$-Thr-Val-Ala-Pro-Val-His-Ile$_{96}$	1000 ± 100	280 ± 30

W.I.; weak inhibition. N.I.; no detected inhibition.

Subscript numbers relate to the position of the peptide within the primary sequence of CRP and bold letters denote amino acid or organic modifications.

MeOSuc is monomethoxy-succinyl, **CBz** is the carbobenzoxy protecting group, **Acetyl-aminocaproic** is 6-acetylamino-N-hexanoyl and **Fmoc** is 9-fluorenylmethoxycarbonyl.

Table 2

Comparison peptides and inhibition constants of hLE

Peptide	Sequence	hLE Ki (μM)
24	Leu-Glu-Ala-Ile-Pro-Met-Ser-Ile (from α_1-PI)	350 ± 70
25	Val_{89}-Thr-Val-Ala-Pro-Val_{94}	900 ± 300
26	Val_{91}-Ala-Pro-Val-His-Ile_{96}	1000 ± 250
27	Val_{89}-Thr-Val-Ala-**(D)Pro**-Val-His-Ile_{96}	560 ± 170
28	Val_{89}-Thr-Val-Ala-**(D)Pro-(D)Val-(D)His**-Ile_{96}	2300 ± 300
29	Val_{89}-Thr-Val-Ala-Pro-Val-**DAB**-Ile_{96}	500 ± 170
30	Val_{89}-Thr-Val-Ala-**Sarcosine**-Val-His-Ile_{96}	1400 ± 500

Subscript numbers relate to the position of the peptide within the primary sequence of CRP and bold letters denote amino acid modifications. **Sarcosine** is N-methyl glycine and **DAB** is 1,4-(L)diaminobutyric acid.

Table 3

Amino acid analysis ratios and HPLC retention time data for peptides 1-30

Peptide **1**. AAA: Thr 1.01(1), Pro 1.01(1), Ala 1.01(1), Val 3.10(3), Ile 1.01(1), His 0.99(1). HPLC: R.T. 37.9 min.

Peptide **2**. AAA: Thr 0.85(1), Ser 0.96(1), Glu 3.13(3), Pro 2.18(2), Gly 0.96(1), Ala 1.02(1), Val 4.08(4), Ile 2.00(2), Leu 1.01(1), Phe 0.98(1), His 0.96(1). HPLC: R.T.51.3 min.

Peptide **3**. AAA: Thr 1.97(2), Ser 1.98(2), Glu 2.64(3), Pro 1.61(2), Gly 2.02(2), Ala 1.04(1), Val 5.11(5), Ile 1.98(2), Leu 1.00(1), Phe 1.99(2). HPLC: R.T. 54.1 min.

Peptide **3a**. AAA: Glu 3.0 (3), Ser 1.9 (2), His 1.0 (1), Gly 2.0 (2), Thr 3.0 (3), Ala 1.0 (1), Cys 0.9 (1), Val 5.0 (5), Ile 2.0 (2), Phe 2.0 (2), Leu 1.1 (1), Pro 2.0 (2). HPLC R.T.= 31.1.

Peptide **3b**. AAA: Glu 4.0 (4), Ser 3.9 (3), His 1.0 (1), Gly 2.0 (2), Thr 3.0 (3), Ala 1.9 (1), Cys 1.0 (1), Val 5.0 (5), Ile 2.1 (2), Phe 2.0 (2), Leu 0.9 (1), Pro 2.0 (2). HPLC R.T.= 32.2

Peptide 3c. AAA: Asp 1.0 (1), Glu 3.1 (3), Ser 2.0 (2), His 1.0 (1), Gly 3.0 (3), Thr 2.9 (3), Ala 1.0 (1), Tyr 0.9 (1), Cys 0.9 (1), Val 5.0 (5), Ile 3.0 (3), Phe 2.0 (2), Leu 1.0 (1), Pro 2.0 (2). HPLC R.T.= 32.3

Peptide 4. AAA: Thr 1.12(1), Pro 0.97(1), Cys 0.98(2), Val 3.46(3), Ile 1.00(1), Leu 1.43(1), Phe 1.36(1) His 1.98(2). HPLC: R.T. 44.8 min.

Peptide 5. AAA: Thr 0.92(1), Ser 0.91(1), Glu 3.00(3), Pro 1.87(2), Gly 0.98(1), Ala 1.00(1), Cys 0.90(2), Val 4.06(4), Ile 1.88(2), Leu 2.21(2), Phe 2.20(2) His 2.05(1). HPLC R.T.= 51.9 min.

Peptide 6. AAA: Val, 2.99(3); Thr, 0.97(1); Ala, 1.01(1); Pro, 1.00(1); His, 0.98(1); Ile, 1.00(1). HPLC R.T.= 26.7 min.

Peptide 7. AAA: Val, 2.98(3); Thr, 0.95(1); Ala, 1.01(1); Pro, 1.00(1); His, 0.97(1); Ile, 0.98(1). HPLC R.T.= 24.6 min.

Peptide 8. AAA: Val, 3.00(3); Thr, 0.96(1); Ala, 1.02(1); Pro, 1.05(1); His, 1.00(1); Ile, 1.04(1). HPLC R.T.= 26.7 min.

Peptide 9. AAA: Val, 3.15(3); Thr, 0.93(1); Ala, 1.01(1); Pro, 1.01(1); Ser, 0.95(1); Ile, 1.00(1). HPLC R.T.= 27.0 min.

Peptide 10. AAA: Val, 3.03(3); Thr, 0.96(1); Ala, 1.03(1); Pro, 1.05(1); Phe, 0.98(1); Ile, 1.01(1). HPLC R.T.= 27.3 min.

Peptide 11. AAA: Val, 2.97(3); Thr, 0.94(1); Ala, 1.03(1); Pro, 1.09(1); His, 0.94(1); Ile, 0,97(1). HPLC R.T.= 24,5 min.

Peptide 12. AAA: Val, 2.98(3); Thr, 0.95(1); Ala, 1.03(1); Pro, 2.04(2); His, 0.96(1); Ile, 0.99(1). HPLC R.T.= 25.3 min.

Peptide 13. AAA: Val, 1.99(2); Thr, 0.96(1); Ala, 1.00(1); Pro, 2.03(2); His, 0.98(1); Ile, 0.94(1); Phe, 1.01(1). HPLC R.T.= 30.0 min.

Peptide 14. AAA: Val, 2.96(3); Thr, 0.95(1); Ala, 1.04(1); Pro, 3.13(3); His, 0.94(1); Ile, 0.95(1). HPLC R.T.= 24.8 min.

Peptide 15. AAA: Val, 3.01(3); Thr, 0.95(1); Ala, 1.03(1); Pro, 0.98(1); His, 1.00(1); Ile, 1.01(1). HPLC R.T.= 20.8 min.

Peptide 16. AAA: Val, 3.02(3); Thr, 0.93(1); Ala, 1.30(1); Pro, 1.02(1); His, 1.01(1); Ile, 1.00(1). HPLC R.T.= 25.3 min.

Peptide **17.** AAA: Val, 2.63(3); Thr, 1.09(1); Ala, 0.99(1); Pro, 1.20(1); His, 1.16(1); Ile, 1.11(1). HPLC R.T.= 29.8 min.

Peptide **18.** AAA: Val, 2.92(3); Thr, 0.97(1); Ala, 0.91(1); Pro, 0.97(1); His, 1.02(1); Ile, 1.00(1). HPLC R.T.= 29.1 min.

Peptide **19.** AAA: Val, 3.03(3); Thr, 0.96(1); Ala, 0.89(1); Pro, 0.90(1); His, 1.03(1); Ile, 0.97(1). HPLC R.T.= 30.1 min.

Peptide **20.** AAA: Val, 3.02(3); Thr, 1.16(1); Ala, 1.29(1); Pro, 1.04(1); His, 1.02(1); Ile, 0.98(1). HPLC R.T.= 29.4 min.

Peptide **21.** AAA: Val, 3.02(3); Thr, 0.95(1); Ala, 0.88(1); Pro, 0.97(1); His, 1.02(1); Ile, 1.00(1). HPLC R.T.= 30.4 min.

Peptide **22.** AAA: Val, 3.01(3); Thr, 0.94(1); Ala, 1.09(1); Pro, 1.01(1); His, 1.04(1); Ile, 1.00(1). HPLC R.T.= 29.1 min.

Peptide **23.** AAA: Val, 2.90(3); Thr, 1.00(1); Ala, 1.00(1); Pro, 1.02(1); His, 1.02(1); Ile, 0.98(1). HPLC R.T.= 31.2 min.

Peptide **24.** AAA: Ser 1.00(1), Glu 0.97(1), Pro 0.94(1), Ala 1.03(1), Met 1.02(1), Ile 2.13(2), Leu 1.06(1). HPLC: R.T. 44.9 min.

Peptide **25.** AAA: Thr 1.00(1), Pro 0.99(1), Ala 1.00(1), Val 2.08(2). HPLC: R.T.30.6 min.

Peptide **26.** AAA: Pro 1.00(1), Ala 1.01(1), Val 2.03(2), Ile 1.00(1), His 0.98(1). HPLC: R.T. 34.5 min.

Peptide **27.** AAA: Val, 3.00(3); Thr, 1.01(1); Ala, 1.03(1); Pro, 1.02(1); His, 0.95(1); Ile, 0.98(1). HPLC R.T.= 29.3 min.

Peptide **28.** AAA: Val, 2.97(3); Thr, 0.96(1); Ala, 1.02(1); Pro, 1.04(1); His, 0.97(1); Ile, 1.00(1). HPLC R.T.= 27.8 min.

Peptide **29.** AAA: Val, 3.09(3); Thr, 0.96(1); Ala, 1.01(1); Pro, 1.01(1); DAB, 1.03(1); Ile, 1.04(1). HPLC R.T.= 26.6 min.

Peptide **30.** AAA: Val, 3.03(3); Thr, 0.95(1); Ala, 1.03(1); Sar, 0.96(1); His, 1.03(1); Ile, 1.01(1). HPLC R.T.= 27.1 min.

HPLC retention times (RT) for peptides **6-23** and **27-30** are given for the following gradient: t=0 min. B=5 %, t=5 min. B=5 %, t=55 min. B=100 %. HPLC retention times for peptides **1-5** and **24-26** are given for the following gradient: t=0 min. B=5 %, t=5 min.

B=5 %, t=60 min. B=70 %. HPLC retention times for peptides **3a-3c** are given for the following gradient: t=0 min. B= 10 %, t=2 min. B= 10 %, t=50 min. B= 90 %.

Example 1.1 Synthesis of core peptide 1

In the synthesis of peptide **1**, H-Val-Thr-Val-Ala-Pro-Val-His-Ile-OH, the standard Fmoc protocol was used as follows:

Peptide elongation cycle:

Step 1. DMF wash	x6
Step 2. Deprotection: 20% piperidine in DMF	x2
Step 3. DMF wash	x6
Step 4. Derivative coupling.	x2

At the end of synthesis:

Step 1. DMF wash	x6
Step 2. Deprotection: 20% piperidine in DMF	x2
Step 3. DMF wash	x6
Step 4. CH_2Cl_2 wash	x6

Deprotection, coupling and wash times and volumes, were calculated by the ABIMED computer program. The resulting lyophilized crude peptide was purified by preparative HPLC to yield approx. 12 mg of lyophilized peptide (white powder), above 99% pure, as determined by its analytical RP-HPLC peak eluting at 37.9 min. Amino acid analysis confirmed the expected sequence, purity, and yield of purified peptide (see Table 3 above).

Example 1.2 Synthesis of peptides 2, 3, 3a-3c, 6-10 and 24-30

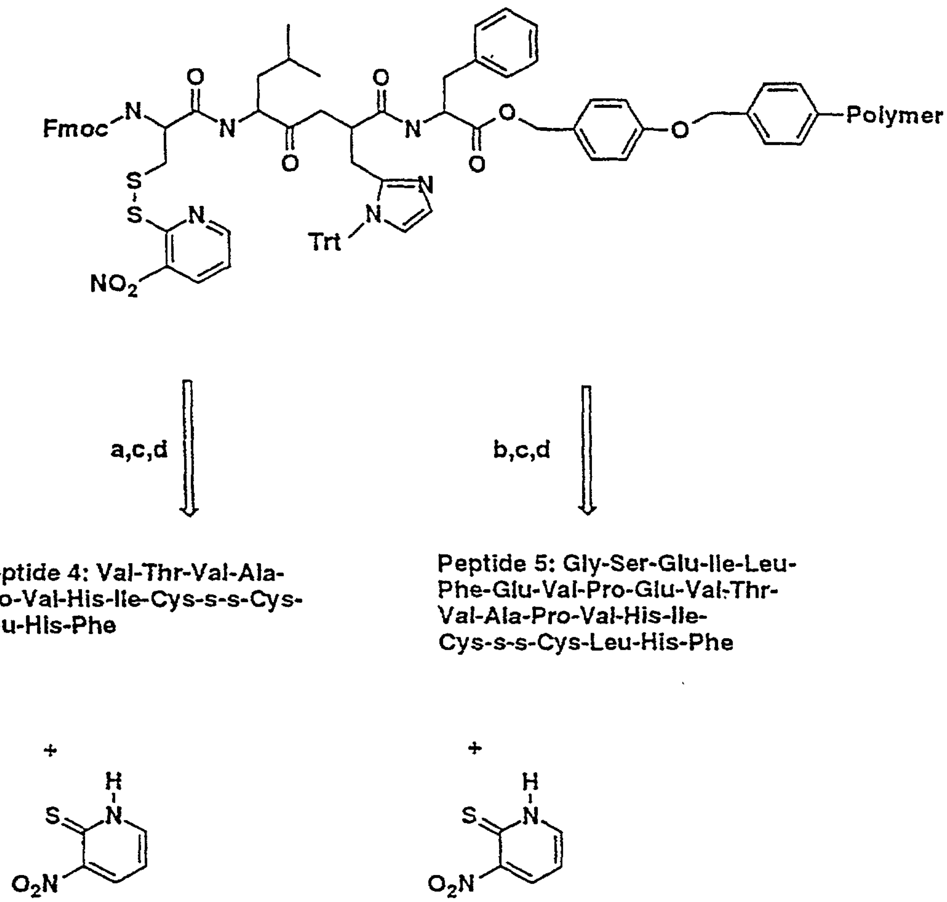
Peptides **2, 3, 3a-3c, 6-10**, and **24-30** were prepared by the standard Fmoc protocol in a similar fashion as described in 1.1 above.

Example 1.3 Synthesis of disulfide bridged peptides 4 and 5

Synthesis of peptides **4** and **5** was carried out according to Scheme 1 hereinafter: the unsymmetrical bridging (oxidation) of two cysteine bonds was performed using a polymer-bound peptidic fragment containing the sulfur-bound NPYS protecting group, which reacts rapidly with the exposed S-H of a pre-purified peptide in solution phase, thus combining the two peptidic fragments.

In the synthesis of Peptide **4**, the peptide H-Val-Thr-Val-Ala-Pro-Val-His-Ile-Cys-OH was prepared using the standard Fmoc protocol, and purified to above 98% by preparative HPLC as described above. The lyophilized peptide (20 mg ≈ 20 μmol) was dissolved in 1 mL N-methyl-pyrrolidone (NMP), and added to 45 mg (≈ 18 μmol) of Fmoc-Cys(NPYS)-Leu-His(Trt)-Phe-Polymer suspended in 1 mL NMP. The combined solution was titrated to apparent basic pH≈8 with 5 % triethylamine in NMP. The reaction mixture was gently rocked for 1 hour at room temperature, and the liberated nitroaromatic compound was observed to yield a dark green color. The polymer was washed thoroughly with NMP followed by CH_2Cl_2. The Fmoc group was removed, and the peptide was cleaved from the polymer as described above. The highest yield was obtained by using non-aqueous NMP at apparent pH 8-8.5 as opposed to reactions carried out in mixed organic aqueous solutions such as DMF/H_2O or CH_3CN/H_2O.

Scheme 1 [a]



[a] Reagents:

a. Val-Thr-Val-Ala-Pro-Val-His-Ile-Cys, NMP.

b. Gly-Ser-Glu-Ile-Leu-Phe-Glu-Val-Pro-Glu-Val-Thr-Val-Ala-Pro-Val-His-Ile-Cys, NMP.

c. Piperidine, NMP.

d. TFA/TES/H_2O.

The analytical RP-HPLC chromatogram of the product was observed to contain a single peak eluting at a longer retention time (51.4 min.) as compared with the precursor reactants. To confirm the integrity of the disulfide bond, 50 μg of peptide was treated with 100 μL of 1 M aqueous 1,4-dithiothreitol (1 hour at pH 8 using 5 % ammonia) to yield the two original HS free-containing fragments exclusively as determined by RP-HPLC co-elution at 37.8 min. and 39.8 min.

In the synthesis of Peptide **5**, the peptide H-Gly-Ser-Glu-Ile-Leu-Phe-Glu-Val-Pro-Glu-Val-Thr-Val-Ala-Pro-Val-His-Ile-Cys-OH was prepared and purified to above 97% by preparative RP-HPLC. The lyophilized peptide (30 mg ≈25 μmol) was dissolved in 1.5 mL NMP, and added to 35 mg (≈14 μmol) of Fmoc-Cys(NPYS)-Leu-His(Trt)-Phe-Polymer suspended in 1 mL NMP and reacted in a similar fashion to peptide **4** as described above with a yield of ≈ 75 %. The analytical HPLC chromatogram of the product contained a single peak eluting at a longer retention time (54.1 min) as compared with the parent reactants. Reducing 50 μg of peptide with 50 μL of 1 M aqueous 1,4-dithiothreitol (1 hour at pH 8 using 5 % ammonia) yields the two original fragments exclusively as determined by RP-HPLC co-elution at 51.9 min. and 39.8 min.

Example 1.4 Synthesis of peptides 11-14

In the synthesis of peptide **11**, H-Val-Thr-Val-Ala-Pro-Val-His-Ile-NH_2, the standard resin was replaced with 12.5 μmols of rink amide solid support [4-2'(4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy-resin] which does not contain the first amino acid. Peptide synthesis was followed in an identical fashion as described in **1.1** above, and upon cleavage from the polymer, the carboxy terminus amidated form of the peptide is obtained. The resulting lyophylized crude peptide was purified by preparative RP-HPLC to yield approx. 8 mg of lyophilized peptide (white powder), above 98% pure, as determined by its analytical RP-HPLC peak eluting at 24.5 min.

Peptides **12**, **13**, and **14** were prepared in an identical fashion.

Example 1.5 Synthesis of peptides 15-16

In the synthesis of peptide **15**, $CH_3OCO(CH_2)_2CO$-Val-Thr-Val-Ala-Pro-Val-His-Ile-OH, monomethyl-succinic-acid was coupled to the exposed N-terminus of H-Val-Thr(*t.*-But)-Val-Ala-Pro-Val-His(Trt)-Ile-Polymer as the final step of solid phase peptide

synthesis. Mono-methyl-succinic-acid (100 μmols, 8 μeqv.), PyBOP (100 μmols), and NMM (200 μmols) were dissolved in 2 mL NMP and added to the resin bound peptide (12.5 μmols) for 1 hour at room temperature followed by extensive flushing with NMP and CH_2Cl_2. The peptide was then cleaved from the polymer and purified by preparative HPLC as described above (sections **i** and **ii** of Materials and Methods), to yield approx. 10 mg of lyophilized peptide (white powder), above 98% pure, as determined by its analytical RP-HPLC peak eluting at 20.8 min.

Peptide **16**, $CH_3OCO(CH_2)_2CO$-Phe-Val-Thr-Val-Ala-Pro-Val-His-Ile-OH, was prepared in an identical fashion using H-Phe-Val-Thr-Val-Ala-Pro-Val-His-Ile-Polymer as the polymer bound peptide.

Example 1.6 Synthesis of peptides 17-18

In the synthesis of peptide **17**, 1-adamantyl-NH-CO-Val-Thr-Val-Ala-Pro-Val-His-Ile-OH, 1-adamantyl isocyanate (100 μmols, 8 μeqv.) was coupled to the N-terminus of H-Val-Thr(*t*-But)-Val-Ala-Pro-Val-His(Trt)-Ile-Polymer (12.5 μmols), as the final stage of solid phase peptide synthesis. The isocyanate compound was allowed to react (without PyBOP or NMM) in 2 mL NMP for 4 hours at room temperature followed by extensive flushing with NMP and CH_2Cl_2. The peptide was then cleaved from the polymer and purified by preparative HPLC as described above (sections **i** and **ii** of Materials and Methods), to yield approx. 10 mg of lyophilized product, above 97% pure, as determined by the analytical RP-HPLC peak eluting at 29.8 min.

In a similar way, Peptide **18**, α-naphtyl-NH-CO-Val-Thr-Val-Ala-Pro-Val-His-Ile-OH, was synthesized using α-naphtyl isocyanate (100 μmols, 8 μeqv.).

Example 1.7 Synthesis of peptide 19

In the synthesis of peptide **19**, $CH_3(CH_2)_6CO$-Val-Thr-Val-Ala-Pro-Val-His-Ile-OH, normal-octanoic acid (100 μmols, 8 μeqv.) was coupled to the N-terminus of H-Val-Thr(*t*-But)-Val-Ala-Pro-Val-His(Trt)-Ile-Polymer (12.5 μmols) as the final step of solid phase peptide synthesis, using PyBOP (100 μmols) and NMM (200 μmols) in 2 mL NMP for 1 hour at room temperature followed by extensive flushing with NMP and CH_2Cl_2. The peptide was then cleaved from the polymer and purified by preparative RP-HPLC as

described above (sections **i** and **ii** of Materials and Methods), to yield approx. 10 mg of lyophilized peptide (white powder), above 98% pure, as determined by its analytical RP-HPLC peak eluting at 30.1 min.

Example 1.8 Synthesis of peptides 20 and 21

In the synthesis of peptides **20** and **21**, carbobenzoxy N-terminus protected amino acids were utilized (50 μmols CBz-Val and 50 μmols CBz-Phe, respectively) as the last amino acid coupling, using identical coupling conditions and 12.5 μmols of polymer, as described in **1.1** above. The carbobenzoxy moiety is stable under peptide-polymer cleavage conditions, which yields the N-terminus derived peptides: CBz-Val-Thr-Val-Ala-Pro-Val-His-Ile-OH and CBz-Phe-Val-Thr-Val-Ala-Pro-Val-His-Ile-OH, respectively.

Example 1.9 Synthesis of peptide 22

In the synthesis of peptide **22**, $CH_3CONH(CH_2)_5CO$-Val-Thr-Val-Ala-Pro-Val-His-Ile-OH, N-acetyl-amino-caproic acid (100 μmols, 8 μeqv.) was coupled to the N-terminus of H-Val-Thr(*t*-But)-Val-Ala-Pro-Val-His(Trt)-Ile-Polymer (12.5 μmols), as the final step of solid phase peptide synthesis, using PyBOP (100 μmols) and NMM (200 μmols) in 2 mL NMP for 1 hour at room temperature followed by extensive flushing with NMP and CH_2Cl_2. The peptide was then cleaved from the polymer and purified by preparative RP-HPLC as described above, to yield approx. 12 mg of lyophilized peptide (white powder), above 97% pure, as determined by its analytical RP-HPLC peak eluting at 29.1 min.

Example 1.10 Synthesis of peptide 23

In the synthesis of peptide **23**, the final step of Fmoc deprotection of the peptide was omitted. The Fmoc moiety is stable under peptide-polymer cleavage and side-chain deprotection conditions, yielding the N-terminus derived peptide: Fmoc-Val-Thr-Val-Ala-Pro-Val-His-Ile-OH.

Example 2. *In-Vitro* inhibition of hLE by peptides 1-30

The hLE inhibitory capability of CRP-derived peptides was evaluated by inhibiting the enzymatic cleavage of MeOSuc-AAPV-NA as described in Materials and Methods (section v). The results are shown in Table 1 for peptides according to the invention and in Table 2 for comparison peptides.

The CRP-derived core octapeptide **1**, Val_{89}-Thr-Val-Ala-Pro-Val-His-Ile_{96}, is shown to be a more potent inhibitor of hLE than the α_1-PI- derived core octapeptide **24**. The inhibitory activity of a peptide with the sequence Val-Ala-Pro-Val is minute (data not shown). The inhibitory activity of the core peptide drops drastically with removal of the residues His-Ile from the carboxy terminus (peptide **25**) or Val-Thr from the amino terminus (peptide **26**). Replacing His_{95} with Ser (peptide **9**) yields inhibitory activity similar to the original peptide while replacement by Phe (peptide **10**) even increases the inhibitory activity.

More insight into the mechanism of inhibition is provided by HPLC time-course elution profiles of peptide **1**, incubated with hLE in PBS (Fig. 2). The peptide is cleaved as predicted exclusively at the Val-His bond generating the expected two fragments, identified by co-elution with peptide **25**. The other bonds in peptide **1** are cleaved only after several days of incubation with the enzyme. No observable cleavage products are detected in the incubation of hLE with peptides **6**, **7** and **8** during the same time-scale (3 hours), indicating that the D analogues are effective in resisting degradation by the enzyme.

The extended sequence of peptide **1** at the amino terminal (peptides **2**,**3**) increases the inhibitory capacity on an equimolar basis. The C-terminus addition of Cys_{97}-Thr_{98} to peptide **3** combined with the addition of N-terminus amino acids Asp_{70}-Ile-Gly-Tyr_{74} (peptide **3c**) increases dramatically the inhibitory activity of Ki= 4 μM towards hLE. Extension of the sequence at the carboxy terminus (peptide **4**) via the disulfide bridge increases to a lesser extent the inhibitory capability.

From the carboxy terminal modifications of the core peptide **1** (peptides **11-14**), -Pro-NH_2 appears to be the most beneficial modification (peptide **12**), increasing substantially the hLE inhibitory capability.

From the amino terminal modifications of the core peptide **1** (peptides **15-23**), methoxysuccinyl-Phe- appears to be the most beneficial modification (peptide **16**), increasing substantially the hLE inhibitory capability.

In contrast, replacement of His_{95} by the charged moiety of diaminobutyric acid (DAB-peptide **29**) reduces dramatically the inhibitory capability. When Pro was replaced by sarcosine (peptide **30**), inhibitory capability was almost completely lost, stressing the structural importance of proline in these CRP-derived peptides.

The hLE inhibitory capability of several peptides derived from various regions within the sequence of CRP were evaluated. No significant inhibition was observed for any of the following peptides: Asn_{160}-Met-Trp-Asp-Phe-Val_{165} , Ser_{18}-Tyr-Val-Ser-Leu-Lys_{23} , Asp_{70}-Ile-Gly-Tyr-Ser_{74} , Val_{153}-Gly-Asp-Ile-Gly-Asn-Val_{159} , Asp_{112}-Gly-Lys-Pro-Arg-Val-Arg-Lys_{119} , Gln_{203}-Leu-Trp-Pro_{206} , Thr_{200}-Lys-Pro-Gln-Leu-Trp-Pro_{206}, Thr_{76}-Val-Gly-Gly-Ser_{80} and Phe_{84}-Glu-Val-Pro-Glu-Val-Thr_{90}.

__Example 3. *In-Vitro* inhibition of hCG by peptides 1-23__

The inhibitory capability of several CRP-derived peptides was evaluated by inhibiting the enzymatic conversion of Suc-AAPF-NA (as described in Materials and Methods (vi) above). The results are shown in Table 1 above. Peptide **1** is slightly inhibitory with a dramatic rise in the inhibitory capacity as the amino terminal is elongated (peptide **3c** is the most prominent). In contrast, peptides **4** and **5** are completely inactive, indicating a poor fit of the disulfide portion of the peptide within the enzyme's subsites.

The most prominent modifications of the core peptide **1** that increase inhibitory activity towards hCG are the aromatic acyl derivatives **18, 21** and **23**.

References

1. Ballue, S.P. & Kushner, I. (1992) C-reactive protein and the acute phase response. *Advances in Internal Medicine,* **37**, 313-336.

2. Bode, B., Meyer, E. & Powers, C.J. (1989) Human leukocyte elastase and porcine pancreatic elastase: X-ray crystal structures, mechanism, substrate specifity and mechanism based inhibitors. *Biochemistry*, **28**(5), 1951-1963.

3. Edwards, P.D., Bernstein, P.R. (1994) Synthetic inhibitors of Elastase. *Med. Res. Rev.* **14,** 127-194 and references cited therein.

4. Finke, P.E. et al., (1995) Orally active β-lactam inhibitors of human leukocyte elastase. 3. Stereospecific synthesis and structure--Activity relationships for 3,3-dialkylazetidin-2-ones. *J. Med. Chem.* **38,** 2449-2462.

5. Gallin, J.I., Goldstein, I.M. & Snyderman, R. (1988) Inflammation. Chapter 41, Pathogenesis of rheumatoid arthritis: A disorder associated with dysfunctional immunoregulation. 751-774, ISBN 008167344-7.

6. Groutas, W.C. (1987) Inhibitors of leukocyte elastase and leukocyte cathepsin G. Agents for the treatment of emphysema and related ailments. *Medicinal Researh Reviews*, 7(2), 227-241.

7. Heck, H.L., Darby, W.L., Bhown, A., Miller, E.J., Bennet, J.C. (1985) Isolation, characterization, and amino terminal amino acid sequence of human leukocyte elastase from normal donors. *Analytical Biochemistry,* **149**, 153-162.

8. Jackson, A.H., Hill, S.L., Afford, S.C., Stockley, R.A. (1984) Sputum soluble phase proteins and elastase activity in patients with cystic fibrosis. *J. Respir. Dis.* **65,** 114-124.

9. Metcalf, J.A., Gallin, J.I., Nauseef, W.M., Root, R.K. (1986) *Laboratory Manual of Neutrophil Function,* Raven Press Ltd., New York.

10. Shephard, E.G., Anderson, R., Rosen, O., Myer, M.S., Fridkin, M., Strachan, A.F. & De Beer, F.C. (1990) Peptides generated from C-reactive protein by a neutrophil membrane protease. Amino acid sequence and effects of peptides on neutrophil oxidative metabolism and chemotaxis. *J. Immunol.,* **145**, 1469-1476.

11. Shephard, E.G., Kelly, S.L., Anderson, R. & Fridkin, M. (1992) Characterization of neutrophil-mediated degradation of human C-reactive protein and identification of the protease. *Clin. Exp. Immunol.*, 87, 509-513.

12. Vachino, G., Heck, L.W., Gelfand, J.A., Kaplan, M.M., Burke, J.F., Berninger, R.W., McAdam, K.P. (1988) Inhibition of human neutrophil and Pseudomonas elastases by the amyloid P-component: A constituent of elastic fibers and amyloid deposits. *J. Leukocyte Biol.* 44, 529-534.

13. Yavin, E.J., Rosen, O., Pontet, M., Shephard, E.G., Fridkin, M. (1995) Proteolysis of human C-reactive protein by neutrophil-derived lysosomal enzymes generates peptides which modulate neutrophil function: Implication to the anti-inflammatory mechanism. *Letters in Peptide Science*, 2, 7-16.

CLAIMS

1. A peptide capable of inhibiting *in vitro* the enzymatic activity of human Leukocyte Elastase (hLE) and/or of human Cathepsin G (hCG), said peptide being selected from:

(i) a core peptide corresponding to positions 89-96 of the sequence of human C-reactive protein (CRP) of the formula:

Val89-Thr-Val-Ala-Pro-Val-His-Ile96

or a modification thereof characterized by:

(ii) substitution of Ile96 by a hydrophobic amino acid residue ;

(iii) substitution of His95 by D-His or by a residue selected from Asp, Glu, Ser, Thr, Phe and Tyr, N-alkyl derivatives thereof and D-forms of the foregoing;

(iv) substitution of Val94 by D-Val, or by a residue selected from Ala, His and Phe, and D-forms of the foregoing;

(v) substitution of Ala92 by a hydrophobic amino acid residue;

(vi) substitution of Val91 by Ala or Gly;

(vii) substitution of Thr90 by a residue selected from Asn, Asp, Gln, Glu, Ala, Val and Pro;

(viii) substitution of Val89 by a hydrophobic amino acid residue;

(ix) a peptide obtained by elongation of a peptide (i) to (viii) at the N- and/or C-terminal;

(x) an amide of the C-terminal of a peptide (i) to (ix); and

(ix) an N-acyl derivative of a peptide (i) to (x).

2. A peptide according to claim 1 wherein the hydrophobic amino acid residue is selected from a residue comprising Leu, Ile, Val, Phe, Tyr, Nle and Nva.

3. A peptide according to claim 1(ix) wherein the peptide is elongated by additional amino acid residues at the N-terminal.

4. A peptide according to claim 3 wherein the additional amino acid residues constitute sequences of the human CRP.

5. An N-acyl peptide according to claim 1(xi) wherein acyl is a radical R-X-CO-, wherein R is substituted or unsubstituted hydrocarbyl and X is a covalent bond, O, NH, or NHCO.

6. An N-acyl peptide according to claim 5 wherein R is optionally substituted alkanoyl or aroyl.

7. An N-acyl peptide according to claim 6 wherein the acyl radical is selected from octanoyl, monomethoxysuccinyl, carbobenzoxy (benzyl-O-CO-), acetylaminocaproyl, Fmoc (fluorenylmethoxycarbonyl), naphthyl-NH-CO- and adamantyl-NH-CO-.

8. A peptide according to any one of claims 1 to 7 selected from the sequences:

Val-Thr-Val-Ala-Pro-Val-His-Ile
Val-Thr-Val-Ala-Pro-Val-(D)His-Ile
Val-Thr-Val-Ala-Pro-(D)Val-His-Ile
Val-Thr-Val-Ala-Pro-(D)Val-(D)His-Ile
Val-Thr-Val-Ala-Pro-Val-Ser-Ile
Val-Thr-Val-Ala-Pro-Val-Phe-Ile
Val-Thr-Val-Ala-Pro-Val-His-Ile-NH_2
Val-Thr-Val-Ala-Pro-Val-His-Ile-Pro-NH_2
Val-Thr-Val-Ala-Pro-Phe-His-Ile-Pro-NH_2
Val-Thr-Val-Ala-Pro-Val-His-Ile-Pro-Pro-NH_2
MeOSuc-Val-Thr-Val-Ala-Pro-Val-His-Ile
MeOSuc-Phe-Val-Thr-Val-Ala-Pro-Val-His-Ile
Octanoyl-Val-Thr-Val-Ala-Pro-Val-His-Ile
Acetylaminocaproyl-Val-Thr-Val-Ala-Pro-Val-His-Ile
Adamantyl-NH-CO-Val-Thr-Val-Ala-Pro-Val-His-Ile
α-Naphthyl-NH-CO-Val-Thr-Val-Ala-Pro-Val-His-Ile

CBz-Val-Thr-Val-Ala-Pro-Val-His-Ile

CBz-Phe-Val-Thr-Val-Ala-Pro-Val-His-Ile

Fmoc-Val-Thr-Val-Ala-Pro-Val-His-Ile

wherein Cbz is carbobenzoxy, MeOSuc is monomethoxysuccinyl and Fmoc is 9-fluorenylmethoxycarbonyl.

9. A pharmaceutical composition comprising a CRP-derived peptide according to any one of claims 1 to 8 and a pharmaceutically acceptable carrier.

10. Use of a CRP-derived peptide according to any one of claims 1 to 8 for the preparation of a pharmaceutical composition for the treatment of chronic inflammatory conditions.

11. Use according to claim 10 wherein the chronic inflammatory condition is rheumatoid arthritis, pulmonary emphysema or cystic fibrosis.

12. A method for the treatment of a chronic inflammatory condition which comprises administering to a patient in need thereof an effective amount of a peptide according to any one of claims 1 to 8.

13. A method according to claim 12 wherein the chronic inflammatory condition is rheumatoid arthritis, pulmonary emphysema or cystic fibrosis.

ABSTRACT

A peptide corresponding to positions 89-96 of the human C-reactive protein (CRP) of the formula: Val$_{89}$-Thr-Val-Ala-Pro-Val-His-Ile$_{96}$ and modifications thereof obtained by substitution, elongation, amidation of the C-terminal or acylation of the N-terminal, inhibit in vitro the enzymatic activity of human leukocyte elastase (hLE) and/or of human leukocyte cathepsin G(hCG) and can be used for the treatment of chronic inflammation conditions such as rheumatoid arthritis, pulmonary emphysema and cystic fibrosis.

APPROVED BY DRAFTSMAN | O.G. FIG. | CLASS | SUBCLASS

09/117380

pGlu-Thr-Asp-Met-Ser-Arg-Lys-Ala-Phe-Val-Phe-Pro-Lys-Glu-Ser-	15
Asp-Thr-Ser-Tyr-Val-Ser-Leu-Lys-Ala-Pro-Leu-Thr-Lys-Pro-Leu	30
Lys-Ala-Phe-Thr-Val-Cys-Leu-His-Phe-Tyr-Thr-Glu-Leu-Ser-Se-	45
Thr-Arg-Gly-Tyr-Ser-Ile-Phe-Ser-Tyr-Ala-Thr-Lys-Arg-Gln-Asp-	60
Asn-Glu-Ile-Leu-Ile-Phe-Trp-Ser-Lys-Asp-Ile-Gly-Tyr-Ser-Phe-	75
Thr-Val-Gly-Gly-Ser-Glu-Ile-Leu-Phe-Glu-Val-Pro-Glu-Val-Thr-	90
Val-Ala-Pro-Val-His-Ile-Cys-Thr-Ser-Trp-Glu-Ser-Ala-Ser-Gly-	105
Ile-Val-Glu-Phe-Trp-Val-Asp-Gly-Lys-Pro-Arg-Val-Arg-Lys-Ser-	120
Leu-Lys-Lys-Gly-Tyr-Thr-Val-Gly-Ala-Glu-Ala-Ser-Ile-Ile-Leu-	135
Gly-Gln-Glu-Gln-Asp-Ser-Phe-Gly-Gly-Asn-Phe-Glu-Gly-Ser-Gln-	150
Ser-Leu-Val-Gly-Asp-Ile-Gly-Asn-Val-Asn-Met-Trp-Asp-Phe-Val-	165
Leu-Ser-Pro-Asp-Glu-Ile-Asn-Thr-Ile-Tyr-Leu-Gly-Gly-Pro-Phe-	180
Ser-Pro-Asn-Val-Leu-Asn-Trp-Arg-Ala-Leu-Lys-Tyr-Glu-Val-Gln-	195
Gly-Glu-Val-Phe-Thr-Lys-Pro-Gln-Leu-Trp-Pro-OH	206

Fig. 1

APPROVED BY DRAFTSMAN | O.G. FIG. | CLASS | SUBCLASS

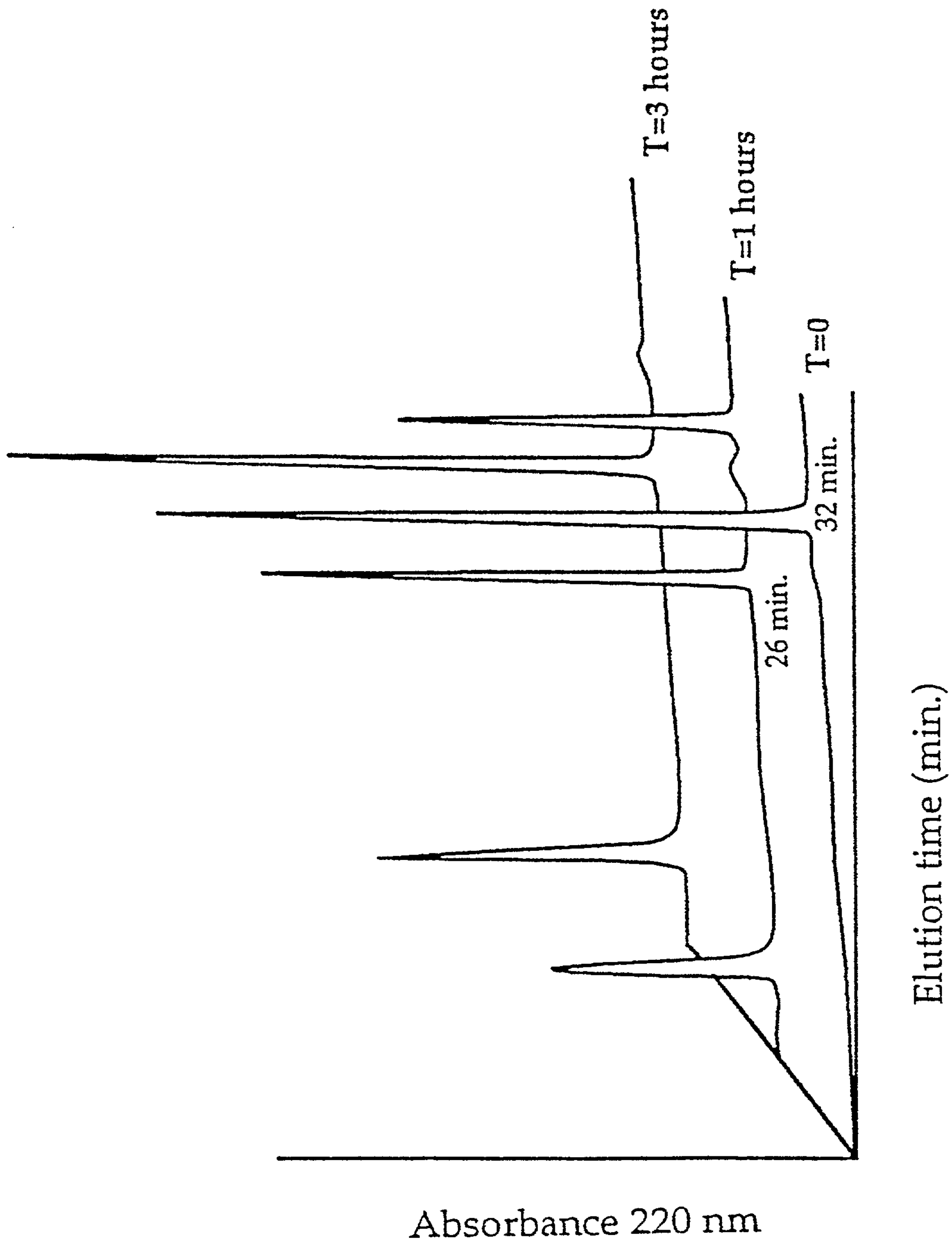


Fig. 2

 [X] Original [ ] Supplemental Atty.Docket: FRIDKIN=1

Combined Declaration for Patent Application and Power of Attorney

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
ANTI-INFLAMMATORY PEPTIDES DERIVED FROM C-REACTIVE PROTEIN

the specification of which (check one)

[ ] is attached hereto;
[ ] was filed in the United States under 35 U.S.C. §111 on __________, as USSN __________*; or
[XX] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international (PCT) application, PCT/IL97/00032; filed January 27, 1997, entry requested on __________*; national stage application received USSN __________*; §371/§102(e) date __________* (*if known),

and was amended on July 29, 1998 __________ (if applicable).

(include dates of amendments under PCT Art. 19 and 34 if PCT)

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119, 365 of any prior foreign application(s) for patent or inventor's certificate, or prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked and have also identified below any such application having a filing date before that of the application on which priority is claimed:

(Number)	(Country)	(Day Month Year Filed)	YES	NO
116976	Israel	January 31, 1996	[ ]	[ ]
			[ ]	[ ]

I hereby claim the benefit under 35 U.S.C. § 120 of any prior U.S. non-provisional Application(s) or prior PCT Application(s) designating the U.S. listed below, or under § 119(e) of any prior U.S. provisional applications listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information as defined in 37 C.F.R. §1.56(a) which occurred between the filing date of the prior application and the national filing date of this application:

(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)

I hereby appoint the following attorneys, with full power of substitution, association, and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

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 Atty.Docket: FRIDKIN=1
Title: ANTI-INFLAMMATORY PEPTIDES DERIVED FROM C-REACTIVE PROTEIN
U.S. Application filed ____________, Serial No. ____________
PCT Application filed January 27, 1997, Serial No. PCT/IL97/00032

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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POST OFFICE ADDRESS		

FULL NAME OF FOURTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		

FULL NAME OF FIFTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		

FULL NAME OF SIXTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		

FULL NAME OF SEVENTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		

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